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SHORT COMMUNICATION

Intestinal amino acid and peptide transporters in broiler are modulated by dietary amino acids and protein

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Abstract This study evaluated the effect of three levels of digestible amino acids (DAA; 100, 107 and 114% of Cobb recommendations) on mRNA abundance of peptide (PepT1) and amino acid (AA) transporters in 480-day-old broilers during prestarter period. Jejunal mRNA levels of the PepT1 and $b^{0,+}$AT increased as DAA level increased from 100 to 114%. The expression of CAT1 mRNA in the jejunum was higher in birds fed 100% DAA diet. The transport systems B^0AT and y^+LAT1 were not affected by the dietary treatments. These results demonstrated that dietary content of protein and DAA differentially affected the expression of intestinal peptide and AA transporters to modulate absorption of peptide and AA in broilers.

Keywords Broiler · Digestible amino acids · Protein · Intestinal peptide and amino acid transporters

Introduction

Genetic progress is one of the main factors responsible for the advances in poultry production. Lys, Met and Thr are not only responsible for the broiler performance and their role in protein synthesis but also are required by different metabolic pathways, act as regulators of gene expression, ion fluxes and enzyme activity (Wu 2009; Fafournoux et al. 2000). Absorption of peptide and AA in the intestine is mediated by several transporters. PepT1 provides transportation for di- and tripeptides. Transporters of Lys, Met and Thr are specific for cationic and neutral AA. Most of these transporters exhibit some substrate overlap. Furthermore, these transporters have different ion dependencies and different mechanisms for transporting of AA (Bröer 2008). AA can regulate the transporters in two ways: specific and nonspecific regulation. Substrates may change the mucosal surface area, composition and fluidity of plasma membrane, paracellular permeability through nonspecific regulation (Ferraris 2000). Changes in the density of transporters in cells is because of changes in protein synthesis or protein degradation or insertion of the specific cytoplasmic transporters into the brush border known as specific regulation (Ferraris and Diamond 1989). Therefore, the transport of AA is a major regulatory step in utilization of protein and AA of feed by poultry. The effects of manipulation of prestarter diet on the expression of transporters in small intestine of chickens have not been documented. Thus, the objective of this study was to verify effect of different level of dietary protein and DAA on mRNA expression of intestinal peptide and AA transporters in broilers.

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Table 1 Composition of the diets (as-fed basis)

Ingredients	Treatments[1]		
	Normal	High	Plenty
Corn	44.33	39.44	34.56
Sunflower oil	2.59	3.36	4.14
Soybean meal	32.89	37.01	41.12
Wheat	15.00	15.00	15.00
DL-Methionine	0.31	0.35	0.39
Lysine-HCl	0.26	0.25	0.25
L-Threonine	0.14	0.15	0.16
Choline chloride	0.05	0.05	0.05
Limestone	2.39	2.37	2.34
NaCl (salt)	0.23	0.24	0.23
Vitamin premix[2]	0.03	0.03	0.03
Mineral premix[3]	0.10	0.10	0.10
Sodium sulfate	0.13	0.12	0.11
Ammonium phosphate	1.54	1.52	1.51
CelloLux[4]	0.01	0.01	0.01
Metabolizable energy, kcal/kg	3000	3000	3000
Crude protein, %	21.40	23.00	24.60
Dig-lysine, %	1.19	1.28	1.37
Dig-Met + Cys, %	0.88	0.95	1.02
Dig-Thr, %	0.80	0.86	0.92
Calcium, %	0.96	0.96	0.96
Available *P*, %	0.48	0.48	0.48

[1]Normal, high and plenty diets contain 100, 107 and 114% of digestible amino acid and protein of Cobb recommendations, respectively, during 1–10 days of age

[2]Vitamin premix provided 1 kg of diet with: vitamin A, 10,800 IU; vitamin D3, 2160 IU; vitamin E, 15 IU; vitamin K3, 1.0 mg; vitamin B1, 4 mg; riboflavin, 5 mg; pantothenic acid 10 mg; niacin, 25 mg; vitamin B_6, 8 mg; folic acid, 0.4 mg; vitamin B_{12}, 0.08 mg; biotin, 0.15 mg

[3]Mineral premix provided 1 kg of diet with: I, 0.35 mg; Se, 0.15 mg; Zn, 40 mg; Cu, 8 mg; Fe, 80 mg; Mn, 100 mg

[4]CelloLux contains cellulase, glucanase and xylanase

Materials and methods

A total of 480-day-old Cobb 500 broilers were housed in 24 cage pens in completely randomized design with eight replicates of 20 birds each. They were arranged in three levels of DAA (100, 107 and 114% of Cobb recommendations) through 1–10 days of age. A fixed proportion of DAA relative to crude protein (CP) was maintained in graded increments of CP from 21.4 to 24.6%. The DAA contents of the ingredients were analyzed using NIRS™ DS2500 FOSS prior to dietary formulation. The formula and chemical composition of the diets are shown in Table 1.

On day 10, one bird from each pen was euthanized and sample from each bird's distal jejunum was dissected, rinsed with PBS, and were placed in RNA later (Invitrogen, Carlsbad, USA). The total RNA was isolated from 200 mg of the jejunum using Trizol reagent (Invitrogen Corp., Carlsbad, CA, USA). One microgram of total RNA was digested using DNase (Thermo Fisher Scientific) for 30 min at 37 °C. The RNA quality was checked by 1.2% agarose gel electrophoresis. cDNA was synthesized from RNA using the Mint-2 cDNA synthesis kit (Evrogen, Russia). To ensure that there was no possible genomic DNA contamination, negative controls for all samples were also prepared by performing reverse transcription without enzyme mix. The following PCR conditions were used: 94 °C for 2 min, and 40 cycles of 94 °C for 15 s, 62 °C for 15 s, 72 °C for 20 s. All PCRs were carried out using an Amplitronyx™ 6 Thermal Cycler (Nyx Technik, Inc. CA).

Primers used in this study were described previously (Gilbert et al. 2008; Zhang et al. 2017) and are shown in Table 2. Each of the cDNA samples was diluted 1:5 prior to RT-PCR, and 1 µL diluted cDNA was used per RT-PCR. RT-PCR was performed in triplicate in 10 µL volume reactions that contained 2 µL SYBR Green Master Mix (Evrogen, Russia), 1 µL each of 2 µM forward and reverse primers, 5 µL H_2O and 1 µL of diluted cDNA using a LightCycler® 96 RT-PCR

Table 2 Sequences of real-time PCR primers used for selected genes

SLC name	Gene	GenBank ID	Primer sequence Sense/antisense
SLC15A1	PepT1	NM_204365.1	CCCCTGAGGAGGATCACTGTTGGCAGTT/CAAAAGAGCAGCAGCAACGA
SLC7A9	$b^{0,+}$AT	NM_001199133.1	CAGTAGTGAATTCTCTGAGTGTGAAGCT/GCAATGATTGCCACAACTACCA
SLC7A1	CAT1	NM_001145490.1	CAAGAGGAAAACTCCAGTAATTGCA/AAGTCGAAGAGGAAGGCCATAA
SLC6A19	B^0AT	XM_419056.4	GGGTTTTGTGTTGGCTTAGGAA/TCCATGGCTCTGGCAGAGAT
SLC7A7	y^+LAT1	XM_418326.4	CAGAAAACCTCAGAGCTCCCTTT/TGAGTACAGAGCCAGCGCAAT
	β-actin	NM_205518.1	GTCCACCGCAAATGCTTCTAA/TGCGCATTTATGGGTTTTGTT

SLC solute carrier family

System (Roche, Swiss). The following protocol was used: (i) pre-incubation program (180 s at 95 °C); (ii) amplification, repeated 40 cycles (15 s at 95 °C, 15 s at 62 °C, 15 s at 72 °C); and (iii) a melting program (10 s at 95 °C, 1 min at 65 °C, 1 s at 97 °C).

Statistical analysis

Data were analyzed using the General Linear Model procedure of SAS Version 9.2 with a completely randomized design. If a significant effect was detected, the differences between treatments were separated using Duncan's multiple range test.

Results and discussion

Our objective was to improve our understanding of the mechanisms involved in the nutritional regulation of peptide and AA transporters' gene expression in broilers. Expression of PepT1 in broilers that were fed 114% DAA diet was greater ($P < 0.01$) as compared with broilers that fed 100 or 107% DAA diets (Table 3). PepT1 provides transportation for AA to the enterocytes in the form of di- and tripeptides. Our result is in agreement with finding of Walker et al. (1998), Shiraga et al. (1999), Chen et al. (2005) and Gilbert et al. (2010). In broilers, an increase in abundance of PepT1 mRNA was reported in the small intestine of chickens fed 18 and 24% CP diets with restricted FI and a decrease in abundance of PepT1 was detected in broilers fed a 12% CP diet (Chen et al. 2005). Gilbert et al. (2010) reported that higher amount of AA in the enterocyte of chickens might lead to a substrate-induced upregulation of PepT1 through an effect on cellular metabolism. Shiraga et al. (1999) in rats showed that higher dietary proteins lead to greater abundance of PepT1 mRNA. Shiraga et al. (1999) reported that the 5′ upstream region of PepT1 in rats contains elements that respond to both peptide and free AA substrates. Thus, expressions of intestinal transporters like PepT1 may be affected by end-products of protein digestion, small peptides and AA, which can lead to a larger intestinal transporter capacity. Treatment of human cell with Gly-Sar or Gly-Gln dipeptides increased PepT1 mRNA abundance (Walker et al. 1998). Furthermore, upregulation of PepT1 by a high-protein and AA diet seems to be a rout to take advantage of the excess substrates. The amount of the changes in PepT1 expression and activity are perhaps reliant on duration of particular dietary manipulation, presence of substrate, AA and peptide concentrations, presence of other components in the intestine that have an effect on digestion and absorption process such as carbohydrates and lipids.

The main cationic AA transporter across the apical membrane of the small intestine is system $b^{0,+}AT$. In epithelial cells $b^{0,+}AT$ functions as an antiporter exchanging Leu for Lys so that the influx of lysine is coupled with the efflux of leucine (Pineda et al. 2004). Results of the present study indicate that the birds fed 100% DAA diet had the lowest abundance of $b^{0,+}AT$ mRNA in the jejunum ($P < 0.01$). García-Villalobos et al. (2012) showed that enrichment of diet with Met, Lys and Thr in pigs increased mRNA abundance of $b^{0,+}AT$. He et al. (2013) demonstrated that the dietary supplementation of Lys in pigs increased the expression of $b^{0,+}AT$. The higher $b^{0,+}AT$ expression because of AA supplementation may be explained as an adaptive response of the epithelial cells to absorb the additional ingested AA (Hatzoglou et al. 2004). Morales et al. (2015) showed that dietary free Lys significantly increased duodenal $b^{0,+}AT$ expression compared with protein-bound Lys in pigs. In our experiment, all diets contained approximately 80% protein-bound Lys and 20% free Lys. Hence, the main difference between diets is their Lys content and it rises by increasing the protein content of the diets. Taken together, these results suggest that mRNA abundance of $b^{0,+}AT$ is dependent on the presence of its substrate and protein-bound Lys exerts a marked effect on $b^{0,+}AT$ expression. Another possible explanation for higher $b^{0,+}AT$ expression could be that free AA uptake may be indirectly regulated by PepT1 activity. It has been reported that exposure of Caco-2 cells to dipeptides under V_{max} conditions of PepT1 stimulated AA transport by the $b^{0,+}AT$ (Wenzel et al. 2001).

Table 3 Effect of different levels of dietary protein and digestible amino acids on mRNA abundance ($\times 10^{-3}$) of peptide and amino acid transporters

Treatments[1]	Transporters				
	PepT1	$b^{0,+}AT$	CAT1	B^0AT	y^+LAT1
Normal	0.71 ± 0.07^b	66.50 ± 23.16^b	1.13 ± 0.34^a	123.33 ± 24.22	2.43 ± 0.90
High	0.77 ± 0.09^b	134.29 ± 32.03^a	0.89 ± 0.28^b	119.00 ± 29.61	2.92 ± 0.77
Plenty	0.91 ± 0.14^a	114.83 ± 28.72^a	0.72 ± 0.17^b	121.67 ± 14.72	2.93 ± 0.70
P value	0.00	0.00	0.01	0.87	0.40
SEM	0.04	19.85	0.15	12.05	0.41

[a,b]Within a same column, means with different superscripts differ significantly ($P < 0.05$)

[1]Normal, high and plenty diets contain 100, 107 and 114% of digestible amino acid and protein of Cobb recommendations respectively, during 1–10 days of age

Lys and Arg are transported by CAT1 which expression is modulated by nutrients, hormones and growth factors (Hatzoglou et al. 2004). The lowered expression of CAT1 in broilers fed 107 and 114% DAA diets ($P < 0.01$) is similar to the response observed by Zeng et al. (2011), García-Villalobos et al. (2012). The lower CAT1 expression observed in the present study may be explained by the adaptive regulation theory (Hatzoglou et al. 2004), which states that sufficient or plenty AA supply inhibits while any AA deficiency stimulates CAT1 expression, this regulation takes place at the level of mRNA stability and synthesis (Majumder et al. 2009). Furthermore, cationic AA concentration on the other side of the membrane highly trans-stimulates CAT1 (Closs et al. 2004). It can be deduced that the intra-enterocyte concentration of the cationic AA is higher in broilers fed 107 and 114% DAA diets due to the higher $b^{0,+}$AT expression, which in turn can decrease CAT1 expression. Consequently, the efflux of cationic AA via CAT1 from the enterocyte seems to be related to influx of AA through $b^{0,+}$AT.

B^0AT is the major transporter of neutral AA in the brush border of the intestine (Bröer 2008). In the present study, expression of B^0AT did not differ between treatments ($P > 0.05$). Morales et al. (2015, 2017) reported that the mRNA abundance of B^0 was not affected in pigs fed either the low- or the high-CP diet. Our finding was in contrast with the result of Zhang et al. (2017) who reported that dietary supplementation of L-Met and DL-Met, resulted in enhanced expression of the B^0AT transporters in intestine of broilers. It should be noted that they investigated the effect of Met supplementation (2.2–3.1 kg/ton) to basal diet deficient in Met, but in the current study, we compared the effect of excess protein and AA with basal diet containing adequate level of all nutrients. Furthermore, in their study Met level increased about 60% but in our experiment AA increased by a maximum of 14%. These observations suggested that increasing the dietary AA level by 14% may not be enough to see differences in B^0AT expression.

Neutral and cationic AA are transported across the basolateral membrane of enterocytes by the y^+LAT1 (Bröer 2008). In the present experiment, the dietary treatment did not affect the expression of y^+LAT1 ($P > 0.05$). Similar results were published by He et al. (2013), Wu et al. (2015) and Morales et al. (2015, 2017) in piglets fed different level of dietary protein.

Conclusion

Taken together, the results discussed in the current study showed that protein and AA, by themselves, can play an important role in the control of gene expression. Increasing dietary content of protein and DAA promotes a higher expression of PepT1, $b^{0,+}$AT and lower expression of CAT1 in jejunum. In addition, excess protein and DAA levels in the diet do not affect the expression of B^0AT and y^+LAT1 in jejunum of broilers.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in the present study involving animals were done so in accordance with the ethical standards of the institution at which the present study was conducted.

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